~~DRAFT~~

this draft is considered to be final.
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8/13/99

DATA EVALUATION REPORT

Fungicide Ro 17-0099/000 (Oxine Copper)

Study Type: 13-week Oral (Dietary) Toxicity in Rats

Prepared for:

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May 1994

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Work Assignment Number: 3-72
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Guideline Series 82-1: Subchronic Oral Toxicity in Rats

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DATA EVALUATION REPORT

STUDY TYPE: Guideline series 82-1, subchronic oral toxicity in rats

CAS NUMBER:

TOX CHEM. NUMBER: 253

P.C. NUMBER: 024002

MRID NUMBER: 429868-01

TEST MATERIAL: Fungicide Ro 17-0099/000 (oxine copper)

SYNONYMS: Copper 8-quinolinolate; bis(8-quinolinolato)copper

SPONSOR: Dr. R. Maag, Ltd., Dielsdorf, Switzerland

STUDY NUMBER: Laboratory Project ID, B-157'249

TESTING FACILITY: F. Hoffmann-La Roche & Co., Ltd.
Basel, Switzerland

TITLE OF REPORT: 13-week Oral (Dietary) Toxicity in the Rat with the Fungicide Ro 17-0099/000 (Oxine Copper)

AUTHORS: S. Buser, PhD; F. Mettler, DVM

REPORT ISSUED: June 4, 1991

EXECUTIVE SUMMARY: Fungicide Ro 17-0099/000 (oxine copper) was fed to male and female Wistar rats for 13 weeks at dietary levels to provide an intake of 0, 30, 100, 300, and 1000/700 mg/kg body weight/day. The top dose was reduced from 1000 to 700 mg/kg/day in week 3 (females) and week 6 (males) because of reduced food consumption, reduced body weight gain, poor general condition, and mortality due to anorexia in both sexes.

At 30 mg/kg/day, there were no treatment-related effects. At 100 mg/kg/day, observations included statistically significant elevated bilirubin, ALT and AST activity in males; increased absolute and relative spleen weights in females; and gross and microscopic pathology (diffuse degeneration, focal necrosis, and extramedullary hemapoiesis) of the liver. At 300 mg/kg/day and above, additional treatment-related changes were reduced food consumption,

water consumption, and body weight gain; generally poor condition; death from anorexia; elevated transaminases, bilirubin, cholesterol, and triglycerides; reduced total protein; statistically significant reductions in relative brain (males), testes, and ovary weights; increased absolute and relative spleen weights (both sexes); reduced absolute liver weights; more frequent and severe microscopic lesions in the liver and kidney (degeneration/necrosis); and lymphoid depletion in the spleen, thymus, and lymph nodes. **The LEL of 100 mg/kg/day is based on statistically significant elevated transaminases (ALT, AST) and bilirubin in males, increased absolute and relative spleen weights in females, and increased incidence of diffuse degeneration, focal necrosis, and extramedullary hemapoiesis in the liver. The NOEL is 30 mg/kg/day.**

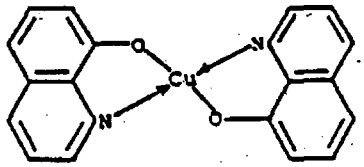
The study is rated Core Minimum, and satisfies the minimum guideline requirements (82-1) for a subchronic oral toxicity study in rats.

A. MATERIALS

1. Test material

Name: Fungicide Ro 17-0099/000 (oxine copper), technical grade active ingredient

Chemical formula: $C_{18}H_{12}CuN_2O_2$



Batch/Lot number: 8293/3

Supplier: Quinoleine, Oissel - France

Purity: 99.5% (re-analysis, February 7, 1990)

Physical properties: Olive green powder

Stability: 2 years in closed container at room temperature

Storage: In the dark at room temperature

Compatibility: Incompatible with metals or aluminum

2. Test animals

Species: Rat

Strain: Kfm Han Wistar

Source: Biological Research Laboratories, Fuellinsdorf, Switzerland

Age and weight at study initiation: Approximately 6 weeks of age; body weights, 170-206 g for males, 143-181 g for females

Housing: Randomly assigned 2/cage in wire-mesh cages

Acclimation period: 7 days after clinical examination

Environmental conditions:
Target temperature: 22 ± 2°C
Target humidity: 55 ± 10%
Photoperiod: 12-hour dark/light cycle
Air changes: 20-25 changes/hour

B. STUDY DESIGN

1. Animal assignment

Animals were randomly assigned to the test groups in Table 1. Treatment lasted 92 days, beginning April 25-26, 1990 and ending June 26-27, 1990.

Table 1. Study Design[a]

Test Group	Target Dose (mg/kg/day)	Number of Rats: Male	Female
A (control)	0	16	16
B (low dose)	30	10	10
C (mid dose)	100	10	10
D (high dose)	300	10	10
E (top dose)	1000/700[b]	16	16

[a]Data were extracted from page 11 of the study report.
[b]The top dose was reduced from 1000 to 700 mg/kg/day at week 3 (females) and at week 6 (males)

Rationale for dose selection: This is a range-finding study for a longer-term carcinogenicity study. The limit dose of 1000 mg/kg/day for carcinogenicity was used.

2. Diet preparation and analysis

The method for preparing test diets was not described. Throughout acclimation and dosing, rats were fed ground KLIBA diet No. 343. Community tap water was available *ad libitum*. Diets were stored at room temperature in closed containers. Homogeneity and stability were tested over two weeks at room temperature, 35, 4, and -20°C. During

the study, samples of treated diet were analyzed once for homogeneity and stability.

Results - In a pre-trial test, the test material was stable in the ground diet for two weeks at room temperature and at 35, 4, and -20°C. Homogeneity of 10, 100, and 1000 ppm dietary samples, prepared 4/3/90, were within 3%, 1%, and 1% of the mean when samples at the top, middle, and bottom of the mix were analyzed. It appears that dietary levels were adjusted weekly to give a constant intake of the test material. Mean compound intake in males was 31 (27-38) mg/kg/day, low dose; 102 (92-125) mg/kg/day, mid-dose; 296 (208-358) mg/kg/day, high-dose; and 747 (479-1208) mg/kg/day, top-dose. Mean compound intake in females was 30 (26-35) mg/kg/day, low-dose; 101 (86-119) mg/kg/day, mid-dose; 299 (252-337) mg/kg/day, high-dose; and 721 (519-1070) mg/kg/day; top-dose. The top-dose level of 1000 mg/kg/day was reduced to 700 mg/kg/day at week 6 in males and at week 3 in females due to generally poor condition, death, and reduced food consumption.

3. Statistics

Rank tests (2-sided Mann-Whitney u-test and Jonckheere test) were used for significance testing of dose-related effects from treatment. In addition, the Kruskal-Wallis Anova test was used for significance testing of water consumption data. Statistical analysis was not performed on mean body weight data or absolute organ weight data.

Organ weight changes were calculated using body-weight (100 g) adjusted organ weights to differentiate between effects on body weight and effects on specific organs. An allometric constant "b," determined empirically for each organ, was used as the adjustment factor:

$$\text{Adjusted organ weight (g)} = \text{absolute organ weight (g)} \times (100/\text{bodyweight})^{b}$$

The following b-values were used: 0.5 for the spleen, adrenal gland, and thyroid gland; 0.0 for the ovaries, testes, and brain; and 0.67 for the heart, kidneys, and liver.

4. A signed and dated Good Laboratory Practice certification statement, a flagging statement, and a list of Quality Assurance Inspections were included.

C. METHODS AND RESULTS

1. Observations

Rats were observed daily for mortality, moribundity, and clinical signs of toxicity.

Results - The incidence of selected clinical signs of toxicity is presented in Table 2. Five top-dose males died during weeks 2-7, and 7 top-dose females died during weeks 3-10. An additional 4 top-dose

TABLE 2. Incidence of Clinical Signs of Toxicity in Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a]

	Incidence in Dose Group:				
	Control 0 mg/kg/day	Low-dose 30 mg/kg/day	Mid-dose 100 mg/kg/day	High-dose 300 mg/kg/day	Top-dose 1000/700[b] mg/kg/day
N =	16	10	10	10	16
Males					
Death	0 (0)[c]	0 (0)	0 (0)	0 (0)	5 (31)
Emaciation	0 (0)	0 (0)	0 (0)	0 (0)	3 (18)
Poor condition	0 (0)	0 (0)	0 (0)	0 (0)	5 (31)
Piloerection	0 (0)	0 (0)	0 (0)	0 (0)	12 (75)
Reduced body temperature	0 (0)	0 (0)	0 (0)	0 (0)	6 (37)
Females					
Death	0 (0)	2 (20)	2 (20)	1 (10)	11 (68)
Cowering	0 (0)	0 (0)	0 (0)	0 (0)	1 (6)
Emaciation	0 (0)	0 (0)	0 (0)	0 (0)	1 (6)
Poor condition	0 (0)	0 (0)	0 (0)	0 (0)	3 (18)
Diarrhea	0 (0)	0 (0)	0 (0)	0 (0)	4 (25)
Piloerection	0 (0)	0 (0)	0 (0)	0 (0)	11 (68)
Reduced body temperature	0 (0)	0 (0)	0 (0)	0 (0)	7 (43)

[a] Data extracted from Tables 9-10, pages 38-47 of the study report (MRID 429868-01)
[b] Dose reduced from 1000 to 700 mg/kg/day at week 3 (females) or week 6 (males)
[c] Numbers in parentheses indicate percentage incidence

females died shortly after blood drawing at the end of the treatment period. Deaths in females occurred after (weeks 3-10) the top-dose was reduced in week 3, while the deaths in males occurred before (weeks 2-7) the top-dose was reduced in week 6. Anorexia from extreme weight loss and generally poor condition were considered the main causes of death.

Emaciation, generally poor condition, reduced body temperature, and piloerection were observed in top-dose animals, although the frequency was usually low (once or twice/animal), and generally occurred midway to late in the study. Most of the top-dose animals were described as within normal limits or in generally good condition (although losing weight) within a week or so of their deaths or the study termination. No treatment-related, biologically significant clinical signs of toxicity were seen at other dose levels, except for the deaths of 2 low-dose, 2 mid-dose, and 1 high-dose female who died shortly after blood drawing.

2. Body weight

Animals were weighed weekly.

Results - Table 3 presents mean body weight and body weight gain data. Body weights of top-dose animals were reduced to 57-75% (males) and 81-91% (females) of controls values. Total body weight gain was significantly ($p \leq 0.01$) reduced in both top-dose (by 80% in males and 73% in females) and high-dose animals (by 37% in males and 22% in females).

3. Food and water consumption

Food consumption (g/animal/day) was measured weekly, except that measurements for weeks 4 and 5 were combined. Water consumption was measured weeks 2-3 and 9-10.

Results - Tables 4 and 5 present mean food and water consumption data. Treatment-related changes (not statistically significant) in top-dose animals included food consumption that was 45-86% (mean 64%) of controls in males and 50-96% (mean 65%) of controls in females. Water consumption was significantly reduced in both sexes, although the reductions were inconsistent across dose levels and varied in significance level throughout the study period.

4. Ophthalmoscopic examination

Eyes of all rats were examined before initiation of dosing and at the end of week 12.

Results - No lesions were observed.

5. Blood was collected at week 7 and week 13 from surviving animals fasted overnight for hematology and clinical chemistry analysis. The checked (X) parameters were examined.

TABLE 3. Mean Body Weight (g ± S.D.) and Body Weight Gain at Representative Intervals for Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a]

Dose Group (mg/kg/day)	Mean Body Weight (g ± S.D.) at Day: 0	14	27	42	56	70	91	Total Body Weight Gain
				Males				
Control (0)	191.5 ± 9.5	265.2 ± 13.4	307.6 ± 19.5	344.1 ± 23.7	366.5 ± 27.6	388.1 ± 29.2	400.7 ± 32.3	209.17 ± 30.00
Low-dose (30)	190.4 ± 8.9	265.9 ± 13.7	311.0 ± 16.9	348.9 ± 18.8	366.6 ± 19.8	383.0 ± 31.8	400.6 ± 20.7	210.17 ± 16.21
Mid-dose (100)	189.2 ± 8.4	259.3 ± 14.2	301.6 ± 18.5	335.1 ± 23.2	359.3 ± 26.7	382.2 ± 26.7	393.0 ± 28.4	203.83 ± 23.95 (93)
High-dose (300)	189.6 ± 8.5 (99)[b]	249.2 ± 16.3 (94)	286.3 ± 24.5 (93)	312.7 ± 28.0 (91)	322.1 ± 32.1 (88)	336.6 ± 31.4 (87)	322.5 ± 35.0 (80)	132.90 ± 27.93** (63)
Top-dose[c] 1000/700	185.7 ± 9.3 (97)	199.7 ± 21.6 (75)	220.5 ± 42.2 (72)	213.8 ± 46.2 (62)	231.8 ± 51.9 (63)	260.4 ± 47.9 (67)	229.9 ± 46.5 (57)	41.39 ± 42.59** (20)
				Females				
Control (0)	156.2 ± 5.9	183.1 ± 8.3	198.4 ± 9.6	207.7 ± 11.7	217.7 ± 11.8	227.3 ± 12.9	224.6 ± 14.2	68.46 ± 11.61
Low-dose (30)	156.5 ± 7.1	180.4 ± 8.2	196.9 ± 8.5	204.6 ± 10.8	214.5 ± 11.9	226.0 ± 14.5	224.2 ± 13.6	67.79 ± 8.08
Mid-dose (100)	154.2 ± 5.6	179.7 ± 8.5	193.9 ± 7.5	202.1 ± 6.7	210.1 ± 5.4	219.6 ± 6.6	216.8 ± 6.5	61.69 ± 4.82 (90)
High-dose (300)	158.3 ± 8.0 (101)	179.0 ± 10.8 (98)	192.4 ± 11.8 (97)	199.6 ± 11.5 (96)	209.6 ± 12.2 (96)	218.0 ± 8.8 (96)	212.0 ± 12.5 (95)	53.07 ± 6.57** (78)
Top-dose[d] (1000/700)	159.4 ± 11.0 (102)	154.7 ± 19.0 (84)	181.2 ± 21.6 (91)	185.7 ± 20.1 (89)	188.7 ± 19.7 (87)	184.8 ± 31.3 (81)	181.7 ± 5.6 (81)	18.68 ± 12.57** (27)

[a] Data extracted from Tables 11-14, pages 50-55 of the study report (MRID 429868-01)
[b] Numbers in parentheses indicate percentage of control
[c] Dose reduced from 1000 to 700 mg/kg/day at week 6
[d] Dose reduced from 1000 to 700 mg/kg/day at week 3

NOTE: It appears that only body weight gain, not mean body weights, were analyzed statistically
$p \leq 0.01$ (Jonckheere-Test)

TABLE 4. Mean Food Consumption (g/animal/day) at Representative Intervals for Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a]

Dose Group (mg/kg/day)	Mean Food Consumption (g/animal/day) at Week: 0 (pretest)	3	5	7	9	11	13	13-week mean ± S.D.
				Males				
Control (0)	17.08	21.63	21.88	20.74	21.78	21.62	19.63	21.14 ± 1.39
Low-dose (30)	17.31	22.27	22.44	19.41	21.44	22.13	19.09	21.24 ± 1.61
Mid-dose (100)	16.96	21.66	21.90	19.76	21.76	21.53	19.40	21.02 ± 1.49
High-dose (300)	16.67 (98)	20.50 (94)	20.08 (92)	14.94 (72)	18.10 (83)	17.31 (80)	11.41 (58)	17.54 ± 2.44 (83)
Top-dose[c] (1000/700)	16.66 (98)	18.66 (86)	13.67 (62)	10.10 (49)	16.39 (75)	12.87 (59)	8.90 (45)	13.45 ± 4.09 (64)
				Females				
Control (0)	13.43	15.66	16.20	14.46	16.18	15.97	13.71	15.45 ± 0.96
Low-dose (30)	13.60	15.43	15.57	13.48	16.18	16.00	13.59	15.16 ± 0.96
Mid-dose (100)	13.11	14.97	15.29	12.90	15.13	14.34	12.89	14.60 ± 0.95
High-dose (300)	13.76 (102)	15.13 (97)	15.36 (95)	13.61 (94)	15.35 (95)	15.24 (95)	12.37 (90)	14.53 ± 0.94 (94)
Top-dose[d] (1000/700)	13.61 (101)	9.06 (58)	15.57 (96)	9.55 (66)	9.48 (59)	7.94 (50)	10.51 (77)	10.01 ± 2.69 (65)

[a] Data extracted from Tables 1-2, pages 30-31 of the study report (MRID 429868-01)
[b] Numbers in parentheses indicate percentage of control
[c] Dose reduced from 1000 to 700 mg/kg/day at week 6
[d] Dose reduced from 1000 to 700 mg/kg/day at week 3

TABLE 5. Mean Water Consumption (g/animal/day) at Representative Intervals for Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a]

Dose Group	Mean Water Consumption (g/animal/day) at Day: 11	14	18	21	56	60	63	67
				Males				
Control	27.72	27.05	29.00	27.15	29.74	26.71	27.31	27.65
Low-dose	30.12	29.05	30.70	30.57	32.81	29.82	31.94**	32.04**
Mid-dose	27.67	26.96	27.99	27.15	30.73	29.28*	30.24	29.79
High-dose	25.41	22.41***	25.99	23.30**	20.65***	19.01***	19.30***	20.12***
Top-dose[c]	27.94 (100)	23.88*** (88)	28.34 (98)	22.95*** (85)	24.67** (83)	20.65*** (77)	20.02*** (73)	19.43*** (70)
				Females				
Control	22.78	21.01	23.57	23.36	27.03	23.90	27.67	25.96
Low-dose	20.57	19.91	20.64	19.75***	25.19	20.62*	23.68**	21.19**
Mid-dose	19.23***	19.42	20.30**	20.37**	22.57***	19.46**	20.60***	20.89**
High-dose	19.38**	18.84	19.77**	21.50	26.68	20.44*	24.90	23.58
Top-dose[d]	28.00*** (123)	15.49** (74)	15.14** (64)	21.40 (92)	24.02 (89)	11.40*** (48)	23.92 (86)	12.64*** (49)

[a] Data extracted from Tables 5-6, pages 34-35 of the study report (MRID 429868-01)
[b] Numbers in parentheses indicate percentage of control
[c] Dose reduced from 1000 to 700 mg/kg/day at week 6
[d] Dose reduced from 1000 to 700 mg/kg/day at week 3
* p ≤0.05 (Kruskal-Wallis Anova + Mann-Whitney-u tests (two sided)
** p ≤0.02 (Kruskal-Wallis Anova + Mann-Whitney-u tests (two sided)
*** p ≤0.002 (Kruskal-Wallis Anova + Mann-Whitney-u tests (two sided)

(a) Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Blood clotting measurements
(Thromboplastin time)
(Clotting time)
(Prothrombin time)
X Leukocyte differential count*
X Mean corpusc. HGB (MCH)
X Mean corpusc. HGB conc. (MCHC)
X Mean corpusc. volume (MCV)
X Reticulocyte count
X Normoblasts

* Required for subchronic studies

Results - Tables 6a and 6b present selected hematology data. Statistically significant changes included reduced lymphocyte counts, which were 97-71% and 107-72% of controls at day 89 in top-dose males and females, respectively. Other statistically significant changes included slightly elevated red blood cell counts, hemoglobin, and packed cell volume (top-dose males at week 7); slightly elevated mean corpuscular volume in top-dose males at week 7; slightly reduced mean corpuscular volume in top-dose females at week 13; slightly reduced (males) and elevated (females) mean corpuscular hemoglobin concentration at week 7; and reduced (males, week 7) and elevated (females, week 13) reticulocytes. However, all the changes appeared to be within the normal range for this species (no historical control data were available). In addition, the changes were variable, inconsistent between males and females, and were probably related to reduced water consumption and dehydration.

(b) Clinical chemistry

Electrolytes

X Calcium*
Chloride*
X Phosphorus*
X Potassium*
X Sodium*
Magnesium*

Enzymes

X Alkaline phosphatase (ALP)
Creatine phosphokinase*
X Serum aspartate aminotransferase (AST)*
X Serum alanine aminotransferase (ALT)*
Lactic acid dehydrogenase (LDH)*
X Cholinesterase (ChE)

Other

X Albumin*
X Globulins
X Blood creatinine*
X Blood urea nitrogen*
X Serum protein electrophoresis
X Glucose*
X Total serum protein (TP)*
X Total bilirubin*
X Cholesterol*
X Triglycerides

* Required for subchronic studies

TABLE 6a. Hematology Data for Male Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a,b]

Parameter	Hematology Data (Mean ± S.D.) in Dose Group: Control 0 mg/kg/day	Low-dose 30 mg/kg/day	Mid-dose 100 mg/kg/day	High-dose 300 mg/kg/day	Top-dose 1000/700[c] mg/kg/day
Red blood cells (10^{12}/L)					
day 43	8.79 ± 0.51	8.61 ± 0.29	8.83 ± 0.36	9.04 ± 0.22	9.10 ± 0.72* (104)
day 89	9.46 ± 0.38	9.35 ± 0.35	9.34 ± 0.39	9.21 ± 1.20	9.57 ± 0.59 (101)
Hemoglobin (mmol/L)					
day 43	9.82 ± 0.44	9.88 ± 0.20	9.92 ± 0.37	10.31 ± 0.26**	10.35 ± 0.74** (105)
day 81	10.32 ± 0.23	10.41 ± 0.25	10.21 ± 0.34	10.14 ± 1.24	10.52 ± 0.65 (102)
Packed cell volume (L)					
day 43	0.49 ± 0.02	0.48 ± 0.01	0.50 ± 0.01	0.51 ± 0.01**	0.52 ± 0.03** (106)
day 89	0.52 ± 0.01	0.52 ± 0.02	0.51 ± 0.01	0.51 ± 0.06	0.53 ± 0.03 (102)
Mean corpuscular volume (fL)					
day 43	55.73 ± 1.49	55.60 ± 1.26	56.30 ± 1.77	57.00 ± 1.76	57.08 ± 2.02* (102)
day 89	54.8 ± 1.81	55.10 ± 0.99	54.90 ± 2.02	55.20 ± 1.32	54.73 ± 1.35 (100)
Mean corpuscular hemoglobin concentration (mmol/L)					
day 43	20.09 ± 0.30	20.80 ± 0.42	19.90 ± 0.32	20.00 ± 0.00	19.92 ± 0.29* (99)
day 89	19.90 ± 0.32	20.10 ± 0.32	19.90 ± 0.32	20.00 ± 0.00	20.00 ± 0.45 (100)
Reticulocytes (10^{-3}/100 WBC)					
day 43	31.55 ± 4.89	33.20 ± 5.37	29.60 ± 6.87	29.90 ± 8.41	26.25 ± 18.24* (83)
day 89	20.10 ± 3.14	31.00 ± 6.86	21.00 ± 8.16	24.00 ± 10.78	27.18 ± 16.45 (135)
Lymphocytes					
day 43	0.79 ± 0.06	0.80 ± 0.07 (101)	0.79 ± 0.06 (100)	0.79 ± 0.06 (100)	0.64 ± 0.11** (81)
day 89	0.77 ± 0.06	0.72 ± 0.06 (94)	0.75 ± 0.06 (97)	0.66 ± 0.13* (86)	0.55 ± 0.18** (71)

[a] Data extracted from Tables 15-18, pages 56-61 of the study report (MRID 429868-01)
[b] Numbers in parentheses indicate percentage of control
[c] Dose reduced from 1000 to 700 mg/kg/day at week 6
* $p \leq 0.05$ (Jonckheere-Test)
** $p \leq 0.01$ (Jonckheere-Test)
# $p \leq 0.05$ (Mann-Whitney-u test)
$p \leq 0.01$ (Mann-Whitney-u test)

Results - Tables 7a and 7b present selected clinical chemistry data. Statistically significant, treatment-related changes were seen in both sexes at the mid-, high- and top-dose levels, and indicated potential liver toxicity. At 13 weeks, AST activity was increased 2 fold and ALT activity was increased 2.8 fold in males receiving 100 mg/kg/day. In males at the 300 mg/kg/day dose, AST activity was increased 7 to 15 fold (weeks 6 and 13) and ALT activity was increased 11-23 fold. No similar trend was seen in females. At 300 and 1000/700 mg/kg/day, elevated bilirubin and reduced total protein were seen in both sexes at week 13. Bilirubin was also elevated in 100 mg/kg/day males at week 13.

6. Urinalysis*

Urine was collected at weeks 6-7 and week 13 from animals in metabolism cages (for 5 hours) after oral administration of water. The checked (X) parameters were examined.

X Appearance (color)	X Glucose
X Volume	X Ketones
X Specific gravity	X Bilirubin
X Ph	X Blood
X Sediment (microscopic)	X Nitrite
X Protein	X Urobilinogen

* Not required for subchronic studies

Results - No treatment-related effects were observed.

7. Sacrifice and Pathology

All animals that died during the study and those sacrificed on schedule were subject to gross pathological examination. The checked (X) tissues were collected from all rats for histological examinations. In addition, double checked (XX) organs were weighed at week 14. Histological examination were limited to the control 700/300 mg/kg/day group, the 300 mg/kg/day group and interim mortalities, gross pathological findings and target organs in the 30 and 100 mg/kg/day groups.

TABLE 7a. Clinical Chemistry Data for Male Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a]

Parameter	Clinical Chemistry Data (Mean ± S.D.) in Dose Group: Control 0 mg/kg/day	Low-dose 30 mg/kg/day	Mid-dose 100 mg/kg/day	High-dose 300 mg/kg/day	Top-dose 1000/700[b] mg/kg/day
Aspartate aminotransferase (µkat/L)					
day 43	1.10 ± 0.09	0.97 ± 0.08	1.20 ± 0.40 (109)	8.19 ± 5.90** (744)	18.14 ± 23.65** (1650)
day 89	1.13 ± 0.17	1.12 ± 0.10	2.30 ± 2.38* (203)	17.27 ± 6.84** (1528)	13.93 ± 10.02** (1230)
Alanine aminotransferase (µkat/L)					
day 43	0.46 ± 0.05	0.28 ± 0.05	0.53 ± 0.27 (115)	5.03 ± 4.57** (1093)	10.09 ± 13.09** (2190)
day 89	0.47 ± 0.09	0.42 ± 0.06	1.33 ± 1.57* (283)	11.06 ± 4.96** (2353)	6.17 ± 3.12** (1310)
Cholinesterase (µkat/L)					
day 43	1.50 ± 0.34	0.99 ± 0.21	1.30 ± 0.14	1.60 ± 0.52 (107)	1.47 ± 0.62 (98)
day 89	1.62 ± 0.33	1.23 ± 0.23	1.42 ± 0.18	1.78 ± 0.51 (110)	1.51 ± 0.66 (93)
Alkaline phosphatase (µkat/L)					
day 43	1.36 ± 0.24	1.33 ± 0.18	1.30 ± 0.15	1.56 ± 0.32 (115)	1.48 ± 0.55 (108)
day 89	0.92 ± 0.12	1.06 ± 0.23	0.98 ± 0.15	1.46 ± 0.37** (159)	1.27 ± 0.13** (138)
Bilirubin (µmol/L)					
day 43	1.26 ± 0.44	1.29 ± 0.40	1.51 ± 0.25 (120)	1.82 ± 0.70* (144)	3.04 ± 1.75** (241)
day 89	1.85 ± 0.37	1.32 ± 0.33	2.17 ± 0.32# (117)	2.34 ± 0.87* (126)	3.13 ± 2.42** (169)
Cholesterol (mmol/L)					
day 43	1.76 ± 0.35	1.84 ± 0.31	1.72 ± 0.33	2.13 ± 0.43 (121)	2.38 ± 0.59** (135)
day 89	1.73 ± 0.32	1.80 ± 0.32	1.77 ± 0.41	2.26 ± 0.41** (131)	2.31 ± 0.41** (134)
Triglycerides (mmol/L)					
day 43	0.70 ± 0.19	0.83 ± 0.25	0.63 ± 0.17	0.62 ± 0.10 (89)	1.04 ± 0.45 (149)
day 89	0.75 ± 0.23	1.05 ± 0.46	0.69 ± 0.23	0.71 ± 0.12 (95)	1.14 ± 0.63 (152)
Total protein (10^9/L)					
day 43	61.47 ± 1.22	60.87 ± 2.32	61.28 ± 1.23	58.11 ± 2.16** (95)	51.05 ± 4.57** (83)
day 89	62.90 ± 1.76	63.58 ± 2.11	63.07 ± 1.15	58.17 ± 1.52** (92)	53.05 ± 5.30** (84)

[a] Data extracted from Tables 19 and 21, pages 68-70 and 74-76, of the study report (MRID429868-01)
[b] Numbers in parentheses indicate percentage of control.

* Significantly different from control, $p \leq 0.05$ using the Jonckheere-Test
** Significantly different from control, $p \leq 0.01$ using the Jonckheere-Test
\# Significantly different from control, $p \leq 0.05$ using the Mann-Whitney U-Test
\## Significantly different from control, $p \leq 0.01$ using the Mann-Whitney U-Test

TABLE 7b. Clinical Chemistry Data for Female Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a]

Parameter	Clinical Chemistry Data (Mean ± S.D.) in Dose Group: Control 0 mg/kg/day	Low-dose 30 mg/kg/day	Mid-dose 100 mg/kg/day	High-dose 300 mg/kg/day	Top-dose 1000/700[b] mg/kg/day
Aspartate aminotransferase (µkat/L)					
day 43	1.12 ± 0.27	1.22 ± 0.43	1.08 ± 0.13	1.37 ± 0.56 (122)	4.91 ± 6.35** (438)
day 89	1.33 ± 0.53	1.10 ± 0.13	1.13 ± 0.16	1.76 ± 0.62* (132)	7.63 ± 3.30** (574)
Alanine aminotransferase (µkat/L)					
day 43	0.41 ± 0.08	0.28 ± 0.07	0.38 ± 0.07	0.48 ± 0.23 (117)	2.45 ± 4.21** (598)
day 89	0.63 ± 0.35	0.37 ± 0.07	0.46 ± 0.12	0.86 ± 0.48 (137)	4.28 ± 3.12** (679)
Cholinesterase (µkat/L)					
day 43	14.57 ± 4.27	14.39 ± 5.08	10.90 ± 2.40 (75)	11.01 ± 2.22* (76)	4.24 ± 2.10** (29)
day 89	18.25 ± 5.21	17.93 ± 4.63	14.04 ± 3.11 (77)	11.61 ± 2.57** (64)	2.51 ± 1.08** (14)
Alkaline phosphatase (µkat/L)					
day 43	0.70 ± 0.16	0.61 ± 0.16	0.67 ± 0.20	0.58 ± 0.10 (83)	0.82 ± 0.26 (117)
day 89	0.41 ± 0.05	0.43 ± 0.11	0.43 ± 0.14	0.38 ± 0.08 (93)	0.85 ± 0.21 (207)
Bilirubin (µmol/L)					
day 43	2.23 ± 0.74	2.42 ± 0.70	1.91 ± 0.92	1.51 ± 0.76* (68)	1.87 ± 1.83** (84)
day 89	1.39 ± 0.61	2.70 ± 0.38	1.25 ± 0.30	1.76 ± 0.79 (127)	2.49 ± 1.03# (179)
Cholesterol (mmol/L)					
day 43	1.60 ± 0.25	1.43 ± 0.56	1.41 ± 0.31	1.49 ± 0.43 (93)	2.24 ± 0.36* (140)
day 89	1.56 ± 0.33	1.59 ± 0.28	1.42 ± 0.32	1.66 ± 0.49 (106)	2.35 ± 0.36 (151)
Triglycerides (mmol/L)					
day 43	0.40 ± 0.09	0.42 ± 0.06	0.48 ± 0.08	0.52 ± 0.06** (130)	0.85 ± 0.34** (213)
day 89	0.44 ± 0.07	0.51 ± 0.08	0.50 ± 0.14	0.60 ± 0.12** (136)	0.89 ± 0.35** (202)
Total protein (10^9/L)					
day 43	67.56 ± 3.10	61.59 ± 3.33	66.90 ± 2.87	59.81 ± 3.65** (88)	51.73 ± 4.42** (77)
day 89	67.91 ± 4.21	65.21 ± 2.73	64.45 ± 4.95	62.77 ± 3.35** (92)	51.10 ± 3.06** (75)

[a] Data extracted from Tables 20 and 22, pages 71-73 and 77-79, of the study report (MRID 429868-01)
[b] Numbers in parentheses indicate percentage of control.
* Significantly different from control, $p \leq 0.05$ using the Jonckheere-Test
** Significantly different from control, $p \leq 0.01$ using the Jonckheere-Test
\# Significantly different from control, $p \leq 0.05$ using the Mann-Whitney U-Test
\## Significantly different from control, $p \leq 0.01$ using the Mann-Whitney U-Test

Respiratory

X Trachea*
X Lung*

Digestive system

X Salivary glands*
X Esophagus*
X Stomach*
X Duodenum*
X Jejunum*
X Ileum*
X Cecum*
X Colon*
X Rectum*
Gall bladder*
X Pancreas*
XX Liver*+

Cardiovasc./Hemat.

X Aorta*
XX Heart*
X Bone marrow*
X Lymph nodes*
XX Spleen
X Thymus*

Urogenital

XX Kidneys*+
X Urinary bladder*
XX Testes*+
Epididymides
Prostate
Seminal vesicle
XX Ovaries*+
X Uterus*

Neurologic

XX Brain*+
X Peripheral nerve*
Spinal cord (3 levels)*
X Pituitary*
X Eyes (optic nerve)*

Glandular

XX Adrenal gland*
Lacrimal gland
Mammary gland*
XX Parathyroids*++
XX Thyroids*++

Other

X Bone*
X Skeletal muscle*
X Skin*
X All gross lesions and masses*
Adipose tissue

* Required for subchronic studies
\+ Organ weight required in subchronic and chronic studies
++ Organ weight required for non-rodent studies

Results -

a. Organ weight. Tables 8a and 8b present selected mean relative and absolute organ weights. Treatment-related, statistically significant ($p \leq 0.01$) effects in 1000/700 mg/kg/day animals include the following: reduced relative (to body weight) brain (males), testes, ovary, and adrenal (females) weights; and increased relative spleen weights (both sexes). Relative spleen weights in high-dose males and high- and mid-dose females were also significantly increased. Absolute organ weights were not summarized or analyzed statistically in the study report, but were tabulated by the reviewer. Absolute spleen weights in females dosed at 100 mg/kg/day and above were 122-143% of controls. In 1000/7000 mg/kg/day animals, absolute liver weights were 71% and 48% of controls for males and females, respectively.

b. Gross pathology. The incidence of selected macroscopic findings are presented in Table 9. Treatment-related findings include an increased incidence of the following in top-dose animals of both sexes: enlargement and thickening of the intestines; focal/nodular changes and discoloration of the liver; and reduced size (males only) of the thymus, lymph nodes, and testes/accessory glands. In addition, the digestive tract of many mid-, high-, and top-dose animals contained a blackish-brown substance, which may have been undigested test material.

c. Microscopic pathology. Table 10 presents the incidence of selected histopathology findings. For both sexes, findings in the liver included

TABLE 8a. Selected Mean Relative Organ Weights (g ± S.D./100 g body weight)[a] in Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[b]

Organ	Mean Relative Organ Weight (g ± S.D/100 g body weight) in Dose Group: Control 0 mg/kg/day	Low-dose 30 mg/kg/day	Mid-dose 100 mg/kg/day	High-dose 300 mg/kg/day	Top-dose 1000/700[c] mg/kg/day
			Males		
Terminal body weight	377 ± 30.9	377.1 ± 19.7	368.7 ± 26.5	305.6 ± 31.0 (81)	215.7 ± 44.9 (57)[d]
Brain	2.047 ± 0.098	1.966 ± 0.053	2.014 ± 0.067	1.989 ± 0.070 (97)	1.848 ± 0.065** (90)
Liver	3.997 ± 0.309	3.992 ± 0.117	4.115 ± 0.410	4.076 ± 0.372 (102)	4.125 ± 0.555 (103)
Kidney	0.907 ± 0.068	0.918 ± 0.074	0.943 ± 0.081	0.942 ± 0.078 (104)	0.915 ± 0.052 (101)
Testes	3.612 ± 0.269	3.443 ± 0.242	3.674 ± 0.265	3.556 ± 0.202 (98)	2.867 ± 0.595** (79)
Spleen	0.351 ± 0.065	0.339 ± 0.038	0.367 ± 0.026 (104)	0.509 ± 0.105** (145)	0.552 ± 0.353** (157)
			Females		
Terminal body weight	211.7 ± 13.5	211.2 ± 12.8	205.0 ± 6.1	201.5 ± 6.1 (95)	169.7 ± 6.4 (80)
Brain	1.868 ± 0.042	1.885 ± 0.066	1.912 ± 0.075	1.871 ± 0.047 (100)	1.775 ± 0.046 (95)
Liver	4.539 ± 1.239	4.238 ± 0.509	4.369 ± 0.374	4.537 ± 0.587 (100)	4.707 ± 1.002 (104)
Kidney	1.082 ± 0.272	0.987 ± 0.101	0.982 ± 0.059	1.009 ± 0.090 (93)	0.996 ± 0.153 (92)
Ovaries	0.155 ± 0.049	0.166 ± 0.025	0.152 ± 0.031	0.131 ± 0.017 (85)	0.092 ± 0.031* (59)
Spleen	0.430 ± 0.115	0.449 ± 0.071	0.534 ± 0.082* (122)	0.534 ± 0.093** (124)	0.687 ± 0.272** (160)
Adrenals	0.047 ± 0.006	0.046 ± 0.004	0.048 ± 0.009	0.045 ± 0.005 (96)	0.036 ± 0.004** (77)

[a] Relative organ weights = absolute organ weights x (100/body weight)^b, where b is an allometric constant (see report text)
[b] Data extracted from Tables 25-26, pages 82-83 of the study report (MRID 429868-01)
[c] Dose reduced from 1000 to 700 mg/kg/day at week 3 (females) or week 6 (males)
[d] Numbers in parentheses indicate percentage of control.
* $0.01 < p \leq 0.05$ (Jonckheere-Test)
** $p \leq 0.01$ (Jonckheere-Test)

TABLE 8b. Selected Mean Absolute Organ Weights (g ± S.D.) in Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a]

Organ	Mean Absolute Organ Weight (g ± S.D.) in Dose Group: Control 0 mg/kg/day	Low-dose 30 mg/kg/day	Mid-dose 100 mg/kg/day	High-dose 300 mg/kg/day	Top-dose 1000/700[b] mg/kg/day
			Males		
Terminal body weight	377.0	377.1 ± 19.7	368.7 ± 26.5	305.6 ± 31.0 (81)	215.7 ± 44.9 (57)[c]
Brain	2.047	1.966 ± 0.053	2.014 ± 0.067	1.989 ± 0.070 (97)	1.848 ± 0.065 (90)
Liver	9.715	9.713 ± 0.485	9.854 ± 1.076	8.615 ± 1.070 (89)	6.863 ± 1.281 (71)
Kidney	2.204	2.236 ± 0.234	2.262 ± 0.253	1.987 ± 0.187 (90)	1.521 ± 0.204 (68)
Testes	3.612	3.442 ± 0.242	3.674 ± 0.265	3.556 ± 0.202 (98)	2.867 ± 9.595 (79)
Spleen	0.672	0.659 ± 0.077	0.704 ± 0.052 (105)	0.888 ± 0.189 (132)	0.814 ± 0.524 (121)
Heart	0.954	1.007 ± 0.086	0.962± 0.081	0.842± 0.088 (88)	0.620 ± 0.087 (65)
			Females		
Terminal body weight	211.7 ± 13.5	211.2 ± 12.8	205.0 ± 6.1	201.5 ± 11.5 (95)	169.7 ± 6.4 (80)
Brain	1.868 ± 0.042	1.885 ± 0.066	1.912 ± 0.075	1.871 ± 0.047 (100)	1.775 ± 0.046 (95)
Liver	6.589 ± 1.719	6.148 ± 0.684	6.259 ± 0.586	6.439 ± 0.850 (98)	6.131 ± 1.313 (48)
Kidney	1.571 ± 0.376	1.432 ± 0.126	1.406 ± 0.080	1.532 ± 0.142 (91)	1.297 ± 0.200 (83)
Ovaries	0.155 ± 0.049	0.166 ± 0.025	0.152 ± 0.031	0.131 ± 0.017 (85)	0.092 ± 0.031 (59)
Spleen	0.626 ± 0.167	0.652 ± 0.107	0.763 ± 0.109	0.757 ± 0.126 (121)	0.896 ± 0.354 (143)
Heart	0.671 ± 0.060	0.685 ± 0.030	0.719 ± 0.055	0.640 ± 0.037 (95)	0.596 ± 0.031 (89)
Adrenals	0.0778 ± 0.0102	0.0764 ± 0.0074	0.0770 ± 0.0134	0.0724 ± 0.0090 (93)	0.0507 ± 0.0045 (65)

[a] Data extracted from Tables 50-51, pages 256-272 of the study report (MRID 429868-01)
[b] Dose reduced from 1000 to 700 mg/kg/day at week 3 (females) or week 6 (males)
[c] Numbers in parentheses indicate percentage of control
NOTE: In the study report, no statistical analysis was performed on absolute organ weights

Table 9. Incidence of Gross Pathology in Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a]

	Incidence in Dose Group:				
	Control 0 mg/kg/day	Low-dose 30 mg/kg/day	Mid-dose 100 mg/kg/day	High-dose 300 mg/kg/day	Top-dose 1000/700[b] mg/kg/day
Males					
Digestive tract					
–black/brown contents	0/16	0/10	5/10	9/10	5/16
Small/large intestine					
–enlargement	0/16	0/10	0/10	0/10	4/16
–thickening	0/16	0/10	0/10	6/10	6/16
Liver					
–focal/nodular changes	0/16	0/10	0/10	0/10	2/16
–discoloration	0/16	0/10	0/10	0/10	4/16
Thymus					
–reduced size	0/16	0/10	0/10	0/10	4/16
Lymph nodes					
–reduced size	0/16	0/10	0/10	0/10	5/16
Testes/accessory glands					
–reduced size	0/16	0/10	0/10	0/10	9/16
Females					
Digestive tract					
–black/brown contents	0/16	1/10	3/10	9/10	9/16
Small/large intestines					
–enlargement	0/16	0/10	0/10	1/10	4/16
–thickening	0/16	2/10	0/10	3/10	11/16
Liver					
–focal/nodular changes	0/16	0/10	0/10	0/10	2/16
–discoloration	0/16	0/10	0/10	0/10	2/16
Thymus					
–reduced size	1/16	0/10	0/10	0/10	0/16
Lymph nodes					
–reduced size	0/16	0/10	0/10	0/10	0/16

[a] Data extracted from the Pathology Report, pp. 299-442 of the study report (MRID 429868-01)
[b] Dose reduced from 1000 to 700 mg/kg/day at week 6 (males) or week 3 (females)

Table 10. Incidence of Histopathology in Rats Fed Fungicide Ro 17-0099/000 for 13 Weeks[a,b]

	Incidence in Dose Group:				
	Control 0 mg/kg/day	Low-dose 30 mg/kg/day	Mid-dose 100 mg/kg/day	High-dose 300 mg/kg/day	Top-dose 1000/700[c] mg/kg/day
Males					
Liver					
-diffuse degeneration	0/10	0/10	5/10 (1.6)	10/10 (3.3)	15/15 (3.7)
-focal necrosis	3/10 (1.0)	2/10 (1.0)	2/10 (1.0)	2/10 (1.0)	6/15 (1.5)
-extramedullary hemapoiesis	0/10	0/10	0/10	1/10 (2.0)	0/15
Spleen					
-lymphoid depletion	0/10	0/10	0/10	0/10	6/15 (4.0)
Thymus					
-lymphoid depletion	0/10	0/10	0/10	0/10	7/13 (4.1)
Lymph nodes					
-lymphoid depletion	0/10	0/10	0/10	0/10	6/13 (2.2)
Kidneys					
-tubular degeneration/necrosis	0/10	0/10	0/10	0/10	12/15 (2.8)
-pigmentation of tubular cells	0/10	0/10	0/10	10/10 (2.4)	14/15 (2.6)
Duodenum					
-villus hypertrophy	0/10	0/10	4/10 (1.5)	10/10 (3.2)	11/12 (2.8)
Bone marrow					
-myeloid atrophy	0/10	0/0	0/0	0/10	9/15 (2.0)
Females					
Liver					
-diffuse degeneration	0/10	1/10 (2.0)	10/10 (1.0)	7/10 (2.0)	15/15 (3.3)
-focal necrosis	9/10 (1.2)	7/10 (1.0)	7/10 (1.4)	8/10 (2.0)	4/15 (3.0)
-extramedullary hemapoiesis	0/10	0/10	0/10	3/10 (1.0)	4/15 (1.3)
Spleen					
-lymphoid depletion	0/10	0/10	0/10	0/10	6/15 (5.0)
Thymus					
-lymphoid depletion	0/10	0/10	0/10	0/10	6/13 (4.3)
Lymph nodes					
-lymphoid depletion	0/10	0/10	0/10	2/10 (2.5)	5/14 (4.4)
Kidneys					
-tubular degeneration/necrosis	0/10	0/10	0/10	0/10	12/15 (2.8)
-pigmentation of tubular cells	0/10	0/10	0/10	2/10 (1.0)	13/15 (1.8)
Duodenum					
-villus hyperplasia	0/10	2/10 (2.0)	3/10 (1.3)	8/10 (2.8)	13/14 (2.4)
Bone marrow					
-myeloid atrophy	0/10	0/2	0/2	1/10 (1.0)	10/16 (2.5)

[a] Data extracted from Table 27, pp. 84-95 of the study report (MRID 429868-01)
[b] Numbers in parentheses indicate average severity, where 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, and 5 = massive.
[c] Dose reduced from 1000 to 700 mg/kg/day at week 6 (males) or week 3 (females)

increased incidence of diffuse degeneration and extramedullary hemapoiesis, generally at the mid-dose level and above. The incidence of focal necrosis was high at all dose levels; however, the incidence was quite high in control animals as well. Increased incidence of lymphoid depletion was observed in the spleen, thymus, and lymph nodes of top-dose males and females. Myeloid atrophy of the bone marrow was also observed in top-dose animals. In the kidney, top-dose animals had tubular degeneration and necrosis, and high- and top-dose animals had pigmentation of the tubular cells.

D. DISCUSSION

The NOEL is 10 mg/kg/day. The LEL of 100 mg/kg/day is based on statistically significant elevated bilirubin and ALT and AST activity in males; increased absolute and relative spleen weights in females; and gross and microscopic pathology of the liver. At dose levels of 300 mg/kg and above, these parameters become more marked and severe, and additional changes become evident (e.g., reduced food and water consumption; reduced body weight gain; generally poor condition; death from anorexia; statistically significant reductions in other organ weights; more frequent and severe microscopic lesions in the liver and kidney; and lymphoid depletion in the spleen, thymus, and lymph nodes). The study authors report that a dose range of 100-300 mg/kg/day would be expected to produce toxicity at a MTD level.

The liver is indicated as a potential target organ, based on elevated transaminase (ALT, AST) levels, reduced total protein levels, and histopathology of diffuse degeneration, focal necrosis, and extramedullary hemapoiesis. Absolute liver weights were reduced in 1000/700 mg/kg/day animals to 71 ad 48% of controls for males and females, respectively.

This study is rated Core Minimum because of the use of inappropriate statistical analysis methods.

E. STUDY DEFICIENCIES

Certain information was missing from the study report, i.e.: the description of the method for preparing test diets; certain clinical chemistry parameters – chloride, magnesium, creatine phosphokinase, and lactic acid dehydrogenase; and histological examinations of the spinal cord and mammary gland. However, these deficiencies in the study would not have affected the interpretation of the study results.

Other, more serious deficiencies include using rank tests instead of ANOVA, which is the preferred method for statistical analysis of continuous data. In addition, statistical analysis of mean body weights and absolute organ weights was not performed.